

Snapshot: DNA Polymerases I Prokaryotes

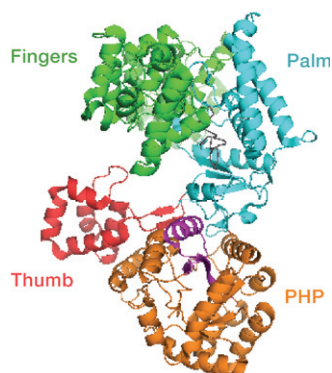
Cell

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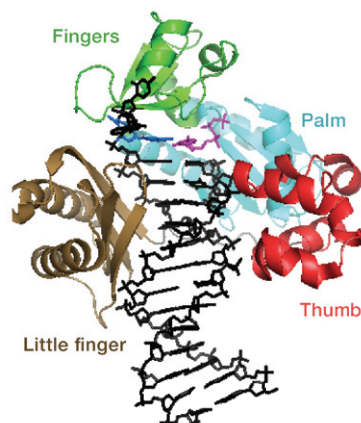
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A Polymerase right hand structure

Pol III (C family)
PDB 2HQA



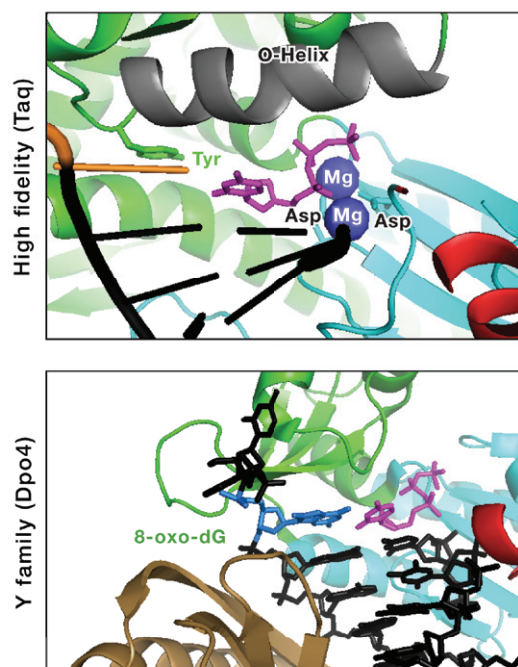
Dpo4
(Pol IV Y family homolog)
PDB 2C2E



B Escherichia coli DNA polymerase domain architecture

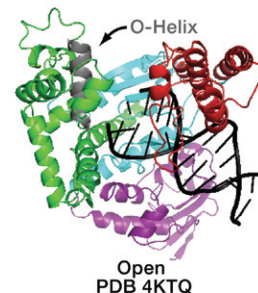


C High-Fidelity versus Y-Family polymerase active site

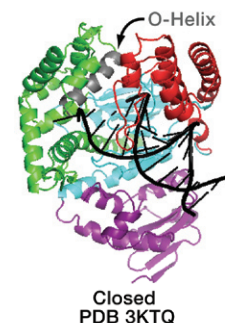


D Open to closed complex

Taq
(Pol I A family homolog)



Open
PDB 4KTQ



Closed
PDB 3KTQ

Polymerase in <i>E. coli</i>	Family	Amino Acid Signature Sequence	Gene Name	Function	Exonuclease Activity	Mutation Rate	Interactions ^a
I	A	R-x(2)-[GSAV]-K-x(3)-[LIVMFY]-[AGQ]-x(2)-Y-x(2)-[GS]-x(3)-[LIVMA]	<i>polA</i>	DNA gaps (repair/maturation of Okazaki fragments)	3' → 5', 5' → 3'	10 ⁻⁵ –10 ⁻⁶	β/γ
II	B ^b	[YA]-[GLIVMSTAC]-D-T-D-[SG]-[LIVMFTC]-[LA]-[LIVMSTAC]	<i>polB</i>	Replication restart, translesion synthesis	3' → 5'	10 ⁻⁵ –10 ⁻⁶ , –2 frameshifts	β/γ
III	C	F-E-[RT]-F-[LMI]-[NSG]-[PF]-[DEKH]-[RG]-[KS]-[MLV]-P-D-[IF]-D	<i>dnaE</i> and <i>dnaQ</i>	Replicative polymerase	None	10 ⁻⁵ –10 ⁻⁶	β/γ
IV	Y	[YFL]-x(2)-Y-x(3)-S-x(2)-[AIV]-x(2)-[IL]-[LFM]-x(2)-[YF]-x(3)-[IVF]-E-x(2)-[SG]-[ILF]-D-E-A-[YF]-[LV]-D-[ILV]-[ST]	<i>dinB</i>	Translesion synthesis	None	10 ⁻³ –10 ⁻⁴ , –1 frameshifts	β/γ/UmuD/RecA
V	Y	[YFL]-x(2)-Y-x(3)-S-x(2)-[AIV]-x(2)-[IL]-[LFM]-x(2)-[YF]-x(3)-[IVF]-E-x(2)-[SG]-[ILF]-D-E-A-[YF]-[LV]-D-[ILV]-[ST]	<i>umuDC</i>	Translesion synthesis	None	10 ⁻³ –10 ⁻⁴ , T to C transitions	β/γ/RecA/UmuD'/SSB/Pol III

^aβ/γ subunit of the Pol III holoenzyme.

^bSignature sequence for this family is from Prositte: <http://us.expasy.org/cgi-bin/nicedoc.pl?PDOC00107>.

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Introduction

The nucleus and mitochondria of eukaryote cells and the nucleoid of prokaryote cells contain remarkable enzymes, called DNA polymerases, which ensure the faithful duplication of genetic material. These enzymatic machines incorporate the building blocks of DNA, deoxyribonucleotide triphosphates (dNTPs), into growing polynucleotide chains. The error rate of these enzymes is astonishingly low with only ~ 1 error for every 10^9 – 10^{10} bases replicated. The first safeguard contributing to this low error rate is the ability of the DNA polymerase to discriminate among incoming dNTPs based on their complementarity to a parental DNA template. However, in the event of misincorporation, many DNA polymerases also have associated “proof-reading” activities that remove an inappropriately added dNTP, thus providing a second safeguard to protect the integrity of the genome. Moreover, cells use a variety of DNA polymerases, called translesion DNA polymerases, whose sole function is to enable recovery from specific genetic insults by endogenous and exogenous mutagens.

Despite this specialization, dNTP incorporation by all DNA polymerases can be described by one basic kinetic model. First, the enzyme (E) binds to the primer template DNA (DNA) to form a binary complex (E:DNA). Then, a dNTP enters the active site of the polymerase to form a tertiary complex (E:DNA:dNTP). Following dNTP binding, the DNA polymerase is activated for catalysis (E':DNA:dNTP), which is thought to occur via a conformational change in the enzyme. Once activated, the enzyme catalyzes phosphodiester bond formation to link the incoming nucleotide with the primer terminus (E':DNA, .PP). The pyrophosphate (PP) is then released, and the polymerase translocates along the template (processive synthesis) or falls off the DNA (distributive synthesis). In this SnapShot, we highlight critical characteristics of DNA polymerases, using the polymerases of the bacterium *Escherichia coli* as an example. A subsequent SnapShot will highlight mammalian DNA polymerases and their roles in disease.

DNA Polymerase Architecture

In addition to having similar kinetic models for the incorporation of dNTPs, all families of DNA polymerases also share similarities in enzyme architecture. The structure of DNA polymerases resemble a human hand, specifically a right hand (panel A, top), which holds and positions the DNA template inside the catalytic site (panel A, bottom). This alignment also helps to ensure the proper selection of the incoming nucleotide. The “right hand” structure can be divided into at least three subdomains: fingers (green), palm (cyan), and thumb (red). Although the location of each subdomain varies in the primary amino acid sequence depending on the polymerase, the position of these subdomains in the structure is similar across the different polymerase families (panels A and B). The highly conserved aspartate residue required for catalysis and the glutamate residue or tyrosine residue (shown as sticks) required for coordinating divalent magnesium ions (blue) and incoming dNTPs (pink) are both located in the palm domain (panel C). The thumb and finger domains make specific contacts with the primer and template DNA strands, respectively (panels A and D).

E. coli Polymerases

In general, DNA polymerases can be grouped into several families (A, B, C, D, X, and Y), based on analyses of their crystal structures and the amino acids in a signature sequence (Table; single letter amino acid abbreviations are used with x indicating any amino acid; numbers in parentheses indicate sequence length; amino acids in brackets indicate that any one of those amino acids is used). Each DNA polymerase family catalyzes replication of different substrates in order to fulfill specialized roles in the cell. *E. coli* cells use five DNA polymerases (Pol I–V) from four different families (A, B, C, and Y) (Table) to ensure faithful replication of its chromosome inside the nucleoid, a nucleus-like region that stores the chromosome but lacks a membrane.

Pol I, the first *E. coli* polymerase discovered, is required for maturation of Okazaki fragments during replication and DNA gap filling during repair and recombination. The 5' to 3' exonuclease subdomain of Pol I is essential for removal of the RNA primer of Okazaki fragments before the polymerase fills in the newly formed gaps. Pol I, like many other high-fidelity polymerases, uses an “induced fit” mechanism to ensure faithful DNA replication (panels C and D). In this mechanism, a large conformational change occurs in the polymerase when the proper nucleotide (pink) pairs with the templating base (orange) (panels C and D). In the absence of nucleotide, the O-helix (gray) stacks with the templating bases in an “open” complex. When the correct nucleotide enters the active site, the O-helix performs a geometric check on the new base-pairing interaction (panel C) while undergoing a large conformational change to reach a “closed” complex formation (panel D).

The major replicative polymerase of *E. coli* is the C family polymerase Pol III, which catalyzes dNTP incorporation at ~ 1000 base pairs per second while making very few replication errors. The Pol III holoenzyme is a multiprotein complex composed of 10 distinct proteins (α , ϵ , θ , τ , γ , δ , δ' , χ , ψ , and β). The catalytic subunit of Pol III is the α subunit, and it is unique to prokaryotes. Nevertheless, Pol III has an active site that is closely related to the active site of Pol β in eukaryotes. The α subunit, encoded by *dnaE*, catalyzes dNTP incorporation without any intrinsic exonuclease activity, which is provided instead by an interaction with the ϵ subunit, encoded by *dnaQ* (Table and panel B). Processivity of the α/ϵ enzyme complex (Pol III in Table and panel B) is enhanced by an interaction with the β (clamp) and the γ (clamp loader) subunits.

Translesion synthesis in *E. coli* is performed by Pol II from the B family and Pol IV and Pol V from the Y family. Pol II is a high-fidelity polymerase that contains intrinsic proof-reading capability. Induced by damaged DNA, Pol II is involved in replication restart and translesion synthesis of abasic sites, interstrand crosslinks, and 3, *N*⁴-ethenocytosine adducts. Despite its intrinsic 3'→5' exonuclease domain, Pol II is capable of forming mutagenic –2 frameshifts after *E. coli* cells are challenged with the carcinogen *N*²-acetylaminofluorene. Pol IV and Pol V are also induced by damaged DNA. However, as members of the Y family of polymerases, Pol IV and Pol V make fewer contacts with the template DNA and incoming dNTPs, and thus, they can accommodate bulky DNA lesions in their active site at the cost of fidelity (panel C). Y-family polymerases also have an extra “little-finger” subdomain, which is thought to provide additional DNA contacts and to contribute to lesion specificity for this class of polymerases (panel A). Pol IV, encoded by *dinB*, efficiently and accurately bypasses adducts on the *N*² position of deoxyguanosine. For example, the Pol IV ortholog Dpo4 is shown bypassing 8-oxo-dG (blue base in panel C). However, Pol IV creates high levels of mutagenic –1 frameshifts on undamaged DNA. Interestingly, the high error rate of Pol IV is implicated in survival under starvation conditions during adaptive mutagenesis. Pol IV activity is modulated by the DNA-damage response proteins UmuD and RecA. Pol V (also known as UmuD'₂C) is the major translesion synthesis polymerase of *E. coli* and is required for genome repair following exposure to multiple types of mutagens, such as UV light, 4-nitroquinoline-1-oxide, and methyl methanesulfonate. UmuC is the polymerase subunit of Pol V, but when it interacts with UmuD, it forms an inhibitory complex that cannot undertake translesion synthesis. RecA then mediates the cleavage of UmuD to UmuD', which leads to activation of the Pol V polymerase.

The fact that the replication and maintenance of the *E. coli* genome involves five distinct DNA polymerases highlights the importance of having specialized polymerases to perform catalysis on specific DNA substrates. Current research is trying to decipher how these various classes of polymerases are regulated to ensure that the proper polymerase gains access to the appropriate primer terminus—a requisite for maintaining genomic integrity while maximizing environmental fitness.

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